Dechlorination of chloroacetanilide herbicides by thiosulfate salts

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Halogenated organic compounds (XOCs) are among the most widely used synthetic chemicals. Many XOCs are recalcitrant to natural degradation and have become prominent environmental contaminants. One group of such XOCs are the heavily used chloroacetanilide herbicides. We have found that chloroacetanilide herbicides are rapidly dechlorinated in water, sand, and soil by thiosulfate salts under ambient conditions. Structural and kinetics analysis suggests that the reaction occurred by S_N2 nucleophilic substitution, in which the chlorine was replaced by thiosulfate and the herbicide was detoxified. Laboratory studies showed that this reaction could be used for removing residues of chloroacetanilide herbicides in water, soil, and sand. Our findings also suggest that some other XOCs may be subject to this reaction. Because common thiosulfate salts are innocuous products (e.g., fertilizers) and the reaction selectively detoxifies XOCs at low thiosulfate levels, this discovery may lead to a new way for safe removal of certain XOCs from the environment.

Halogenated organic compounds (XOC, where X denotes Cl, Br, or I) are among the most widely used man-made chemicals. A great number of these compounds—such as the chlorinated solvents, polychlorinated benzenes (PCBs), chlorofluorocarbons (CFCs), fumigants, and pesticides, among many others—are also notorious environmental contaminants. Many of these compounds are resistant to natural degradation in the environment, and a great effort is being made to prevent further pollution and to restore environmental systems that are already polluted from previous uses (1). Currently, however, there are few safe and effective decontamination methods. Extensive research is being conducted to explore the use of microorganisms to degrade these compounds (1–4). Degraders are rare for many compounds, and for those that do exist, release of microbes into unconfined systems, e.g., aquifers that serve as water supplies, may rouse public concerns (3). Treatments based on chemical oxidation reactions, such as oxidation with hydrogen peroxide, potassium permanganate, or ozone, and physical means, such as solvent flushing and thermal treatments, are nonselective and may, therefore, damage the environmental systems that receive the treatment. Thus, there is a great need to initiate new and selective approaches to decontaminate these compounds.

Here, we report the discovery of nucleophilic dehalogenation of a number of XOCs by thiosulfate salts and the promise of using this reaction for detoxifying these XOCs in environmental matrices. We initially observed rapid reactions between thiosulfate salts and halogenated fumigants that are aliphatic hydrocarbons with Br, Cl, or I substitution (5–7). These fumigants include methyl bromide (CH_3Br), 1,3-dichloropropene ($C_3H_4Cl_2$), chloropicrin (CCl_2NO_2), methyl iodide (CH_3I), and propargyl bromide (C_3H_3Br). Amendment of ammonium thiosulfate (ATS) or sodium thiosulfate (STS) in water or soil at low concentrations enhanced the dissipation of fumigants by one to several orders of magnitude under ambient conditions. Bacterial bioassays showed that the dehalogenation reaction yielded products of little or no acute toxicity and of no detectable mutagenicity (7, 8). We further demonstrated the use of this reaction for reducing fumigant emissions (5), disposing of carbon traps, and removing fumigant residues from soil (6–8).

Our recent exploration led to the discovery that another group of chemicals, the chloroacetanilide herbicides, are susceptible to a similar reaction. Chloroacetanilide herbicides such as alachlor (2-chloro-2′,6′-diethyl-*N*-methoxymethyl-acetanilide), metolachlor [2-chloro-6′-ethyl-*N*-(2-methoxy-1-methylethyl)acet-*o*-toluidide], and acetochlor (2-chloro-*N*-ethoxymethyl-6′-ethylacet-*o*-toluidide) are primary herbicides, and >50 million kg have been used annually in the United States over the last 20 years (9). Because of their relatively high solubility in water and long persistence in soil, residues of these herbicides or their metabolites have been detected in surface and ground water throughout the United States (10–12). These herbicides are classified as B2 carcinogens by the U.S. Environmental Protection Agency, and, therefore, their removal from the environment is of great importance. Our observations also suggest that thiosulfate may react with many more XOCs that have halide substitution on aliphatic carbons, making the reaction broadly useful for environmental pollution control.

Materials and Methods

Chemicals and Test Organisms. Alachlor, acetochlor, metolachlor, and propachlor (2-chloro-*N*-isopropylacetanilide) were obtained from Chem Service (West Chester, PA), and the chemical purity of each compound was >97.5%. STS (purity >99%) and ATS (purity >99.5%) were purchased from Fluka. The test organism used in the acute toxicity assay was the luminescent bacterium *Vibrio fisheri* purchased from AZUR Environmental (Carlsbad, CA). The *Salmonella typhimurium* strains TA97a, TA98, TA100, and TA102 used in the mutagenicity test were obtained from the Division of Biochemistry and Cell Molecular Biology, University of California, Berkeley. Rat hepatic fractions (S9) were purchased from BioReliance (Rockville, MD).

Aqueous System Experiments. A series of aqueous phase experiments were conducted to understand the reaction kinetics between the herbicides and thiosulfate salts and to obtain information on the identity of reaction products. In the first experiment, disappearance of herbicides was determined by HPLC in aqueous solutions of thiosulfate at various concentrations. Solutions of ATS or STS were prepared with deionized water in 100-ml volumetric flasks at 0, 0.1, 0.2, 0.5, 2.0, and 10.0 mM. Stock solutions of herbicides (10.0 mM) were made in methanol. To initiate the reaction, 2.0 ml of each herbicide solution was separately added into the volumetric flasks, and the

Abbreviations: XOC, halogenated organic compounds; STS, sodium thiosulfate; ATS, ammonium thiosulfate; IC, ion chromatography.

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volume was brought to the 100-ml mark with deionized water. The initial herbicide concentration was, therefore, 0.2 mM. The flasks were immediately transferred to and kept in a 20°C incubator under darkness. At 0, 8, 24, 48, 96, and 172 h after the initiation of reaction, three 1.5-ml aliquots were removed from each flask and transferred to 2-ml amber glass vials. A 10-μl aliquot was injected into a Hewlett Packard HP-1090 HPLC for herbicide quantification. Herbicides were eluted through a 250 × 4.6 mm (i.d.) reverse-phase column (Adsorbosphere HS C_{18} 5 μm; Alltech, Deerfield, IL) and detected with a diode-array UV detector. The wavelength of detection was 230 ± 15 nm. The mobile phase was composed of acetonitrile and water, which was acidified to pH3 with phosphoric acid. The percentage of acetonitrile was 85% for metolachlor, 75% for alachlor and acetochlor, and 65% for propachlor. The flow rate of mobile phase was 1.0 ml/min^{-1}, and the temperature of the column was kept at 40°C.

To follow the liberation of chloride (Cl^-) from the reaction, additional aliquots were removed from the flasks, and Cl^- concentration was measured on a Dionex DX-100 ion chromatograph (IC). An aliquot of 100 μl was injected by using an AS40 autosampler, and Cl^- was eluted through an AS-14 column (Dionex) for separation. The mobile phase was made of 3.5 mM Na_2CO_3 and 1.0 mM $NaHCO_3$, and the flow rate was 1.0 ml/min^{-1}.

Aliquots of the reaction mixes also were analyzed by liquid chromatography-mass spectrometry. The analysis was made by direct injection of 50 μl of aqueous samples on a single-quadrupole Micromass ZMD-2000 system (Micromass, Manchester, U.K.). The mobile phase consisted of 90% water and 10% acetonitrile, with a linear ramp to 20% water and 80% acetonitrile by 23 min at a flow rate of 0.2 ml/min^{-1}. The column was a 15-cm C_8 column with an internal diameter of 2.1 mm and a particle size of 5 μm. The drying gas was nitrogen, and data were collected in full-scan ion mode in the 50–400 m/z range and in selected ion-monitoring mode.

Toxicological Assays. Bacteria-based assays were conducted to obtain preliminary information on toxicological alterations from the reaction. The Microtox method was used for acute toxicity assay (13). Herbicides and STS were reacted in 2% NaCl solution until completion, and the reaction mixtures were exposed to the revived luminescent bacteria (*Vibrio fisheri*) for 5 min in cuvettes. The luminescence was recorded at 490 nm with an SLM spectrofluorometer (SLM-Aminco, Urbana, IL). Toxicity was indicated by quenching of the luminescence emitted by the bacteria. Bacterial EC_{50} was estimated by plotting the relative light intensity against analyte concentration after logarithmic conversion and solving for the concentration at which 50% reduction in luminescence occurred. Three replicates were used for each concentration step, and blank controls (i.e., 2% NaCl) were run concurrently with samples.

Mutagenic activity was assayed by using *Salmonella typhimurium* strains TA97a, TA98, TA100, and TA102 by following the Ames method (14). Herbicides and STS were reacted in aqueous solution to completion. Bacterial strains were preincubated with the reaction mixtures for 20 min, with or without addition of the rat hepatic fraction S9. The samples were further incubated at 37°C for 2–3 days in Petri dishes containing minimal nutrition media. Values of mutagenicity ratio (MR) were obtained by dividing the number of revertant colonies in sample plates by that in the blank control. An MR > 2 with a clear dose response was defined as mutagenic, whereas an MR < 2 was defined as a lack of mutagenicity.

Decontamination Experiment. An experiment was carried out under simulated conditions to demonstrate a potential application of the reaction—decontamination of a herbicide-polluted soil or sandy aquifer. A sandy loam soil or silica sand was packed into PVC cylinders 30 cm (length) by 6 cm (i.d.), and the columns then were saturated with water. A pulse of 2.0 mg of herbicide was injected into the columns from the top end. In one group of columns, 20 ml of solution containing 100 mg of STS was injected, whereas in another group of columns, 20 ml of water was injected. The columns were kept at 20°C for one week, and then water was applied to the top end of the column at 1.0 ml/min^{-1}, and the leachate was collected from the bottom end. The collected leachate samples were analyzed for herbicides by HPLC under conditions given above.

Kinetics Modeling. The chemical reaction between the chloroacetanilide herbicides and thiosulfate salts is presumably one of S_N2 nucleophilic substitution, the kinetics of which typically follow a second order relationship which may be expressed as

$$\frac{dC}{dt} = -\mu XC = -\mu(C - C_0 + X_0)C$$

where C and X are the herbicide and thiosulfate concentration, respectively, C_0, X_0 their initial values, and μ the second-order rate constant. Eq. **1** has the solution (15)

$$C(t) = C_0 \frac{(X_0 - C_0)\exp[-\mu(X_0 - C_0)t]}{X_0 - C_0\exp[-\mu(X_0 - C_0)t]}.$$

Note that Eq **2**. has two special limits:

$$C(t) \rightarrow C_0\exp(-\mu X_0 t); \quad X_0 \gg C_0$$

$$C(t) \rightarrow \frac{C_0}{1 + \mu C_0 t}; \quad X_0 \rightarrow C_0$$

To test this reaction hypothesis, we fitted our measurements from the aqueous-phase experiments to Eq. **2**. (or Eq. **3b** when $X_0 = C_0$).

The second-order reaction described in Eq. **1** does not decay as a single exponential and thus does not have a true half life. However, we can characterize the speed of the reaction by defining a 50% disappearance time τ for a given set of conditions as the time when $C/C_0 = 0.5$, which is given by

Table 1. Effective half lives $T_{1/2}$ (d) and pseudo-first-order rate coefficient $k = \mu X_0$ (d^{-1}) for the disappearance of different chloroacetanilide herbicides in water, 2.0 and 10.0 mM ammonium thiosulfate (ATS) solutions at 20°C

	Water		2 mM ATS		10 mM ATS	
Herbicide	k	$T_{1/2}$	k	$T_{1/2}$	k	$T_{1/2}$
Alachlor	<0.01	>100	0.28 ± 0.03	2.5 ± 0.3	1.5 ± 0.07	0.5 ± 0
Acetochlor	<0.01	>100	0.20 ± 0.02	3.4 ± 0.3	1.1 ± 0.05	0.6 ± 0
Metolachlor	<0.01	>100	0.04 ± 0	20.9 ± 2	0.21 ± 0.01	3.4 ± 0.2
Propachlor	<0.01	>100	0.53 ± 0.08	1.3 ± 0.2	3.1 ± 0.07	0.2 ± 0

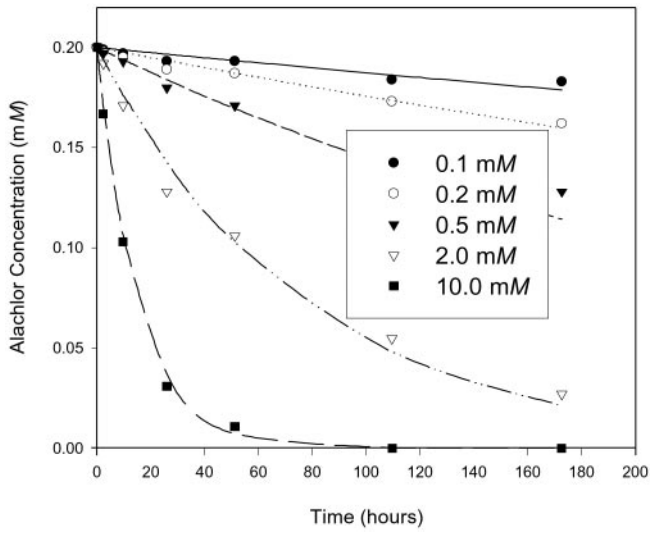

Fig. 1. Dissipation of alachlor (0.2 mM) in solutions of varying STS concentrations at 20°C. Symbols are measured data points, and curves are fitted lines by using the second-order kinetics model.

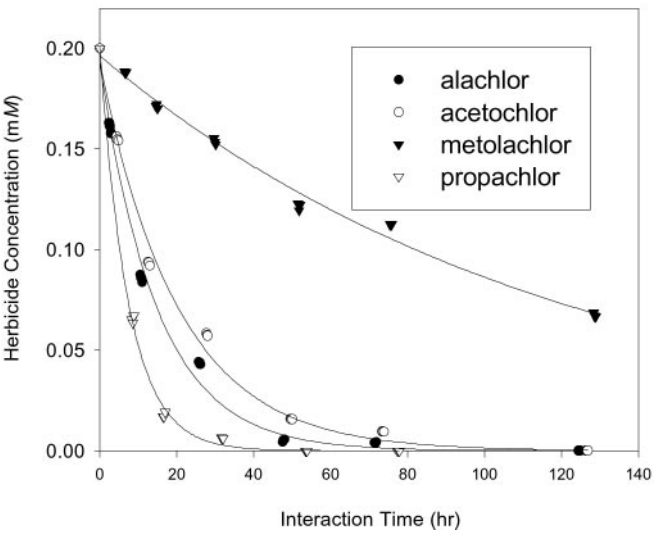

Fig. 2. Disappearance of chloroacetanilide herbicides in 10 mM STS solutions at 20°C. Symbols are measured data points, and curves are fitted lines.

$$\tau = \frac{\ln(2-f)}{\mu X_0(1-f)}; \quad f = \frac{C_0}{X_0} \neq 1$$

$$\tau = \frac{1}{\mu X_0}; \quad C_0 = X_0$$

For $f \leq 0.1$, the pseudo-first-order reaction may be used to define a half-life $T_{1/2}$ with an error of only a few percent or less in estimating the 50% disappearance time. Thus, for high thiosulfate concentrations compared with herbicide concentrations, $\tau \sim T_{1/2} = \ln(\mathbf{2})/\mu X_0$. Obviously, if $f > 2$, the final concentration will be greater than 0.5 C_0.

Results and Discussion

Reaction Kinetics. In water blanks, all chloroacetanilide herbicides showed great stability, and the half-life ($T_{1/2}$) for each herbicide was >100 days (Table 1). This observation is consistent with other researchers' findings (16) and is the cause for the long persistence of these herbicides in environments such as aquifers. The addition of ATS or STS, however, resulted in the rapid disappearance of these compounds from the aqueous system (Table 1), and there was no difference between ATS and STS in causing the dissipation. At an initial thiosulfate concentration of 2 mM, the $\tau \sim T_{1/2}$ of propachlor, alachlor, and acetochlor was shortened to a few days. When the initial thiosulfate concentration was increased further to 10 mM, the $T_{1/2}$ of propachlor, alachlor, and acetochlor decreased to <1 day. Under these conditions, thiosulfate salts caused reduction of the aqueous persistence of each chloroacetanilide herbicide by one to several orders of magnitude.

Table 2. Comparison between the 50% disappearance time τ for alachlor at various initial thiosulfate concentrations X_0 estimated from the second order kinetics solution Eq. 4 and the effective half-life $T_{1/2}$ calculated by assuming pseudo-first-order kinetics

X_0	τ, hr	$T_{1/2}$, *hr*	Error, %
0.2	769.2	533.2	30.7
0.5	241.0	213.3	11.5
2.0	54.9	53.3	2.8
10.0	10.7	10.7	0.6

The effect of different initial concentrations of thiosulfate on the rate of herbicide disappearance is illustrated for alachlor in Fig. 1. The curves represent the simultaneous fitting of the second-order kinetics model Eq. **1** to all of the data, which produced an outstanding agreement ($r^2 = 0.995$) with $\mu = 0.0065$ mM^{-1}/hr^{-1} (or 1.1×10^{-7} M^{-1}/s^{-1}). This exercise validated that the reaction between chloroacetanilide herbicides and thiosulfate salts followed the second-order kinetics that are characteristic of S_N2 nucleophilic substitution reactions. Table 2 summarizes the 50% disappearance time for all of the alachlor treatments together with an estimate of the error involved in assuming pseudo-first-order kinetics.

At the same initial thiosulfate concentration, the herbicides exhibited different reactivity toward thiosulfate (Fig. 2). The relative reactivity followed the order propachlor > alachlor > acetochlor > metolachlor. This dependence suggests that the substitutions at the nucleophilic center, i.e., the chlorinated carbon, influenced the reactivity. Under the condition that the

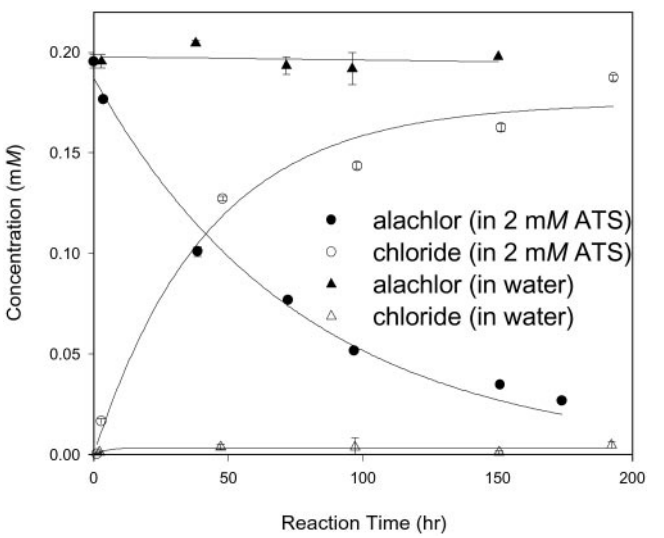

Fig. 3. Consumption of alachlor and liberation of chloride in 2 mM ATS solution at 20°C. The error bars are SD and, where they are not visible, are smaller than the symbols.

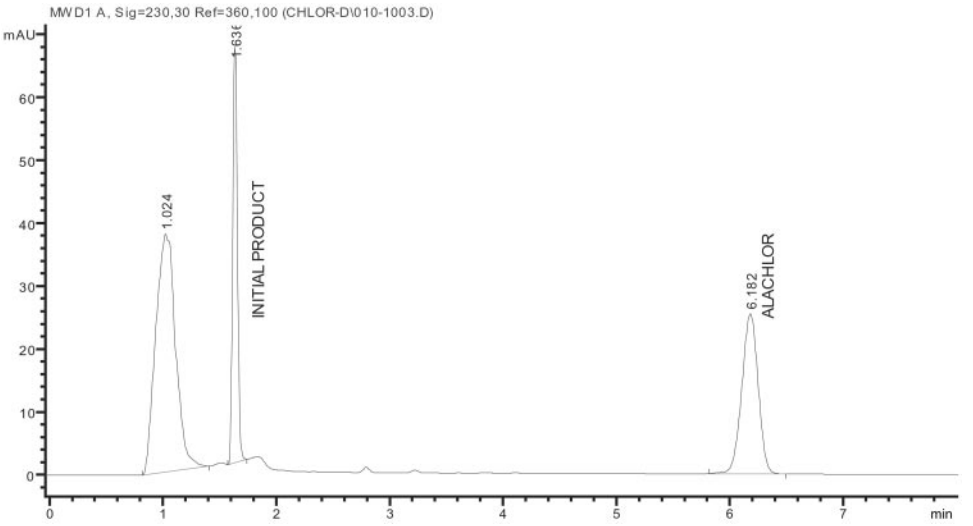

Fig. 4. HPLC chromatogram of alachlor (0.2 mM) and ATS (2 mM) after 38 h of reaction at 20°C.

reaction followed the S_N2 pathway, the nucleophile ($S_2O_3^{=}$) would attack the primary carbon from a direction opposite from the chlorine. The bulky substitutions at this position may have resulted in greater steric hindrance for metolachlor, rendering its primary carbon less accessible by $S_2O_3^{=}$. This analysis also provides a useful lead for identifying XOCs that are easily susceptible to thiosulfate substitution in future research.

Reaction Pathways. Analysis of reaction mixtures by IC showed that as each herbicide reacted with thiosulfate, Cl^- was liberated into the solution. The rate of Cl^- release always equaled the rate of herbicide consumption, as shown in Fig. 3 for alachlor. This finding provided direct evidence that Cl was displaced from the herbicide molecule during the reaction, indicating that the reaction was a stoichiometric substitution.

As the reaction proceeded, a major metabolite peak was invariably detected by HPLC and increased in size with time. Moreover, the retention time suggested a polar product (Fig. 4). The reaction products were further analyzed by LC-MS, and the deprotonated molecular ions were detected after electrospray ionization in the negative mode. The analysis invariably showed the presence of a predominant ion fragment that had a mass identical to the dechlorinated and thiosulfate-substituted herbicide anion.

From the observations that the reaction followed second-order kinetics, and that Cl^- and dechlorinated herbicide-thiosulfate derivative were formed, it may be concluded that the reaction followed a pathway as depicted for alachlor in Fig. 5*A* or for XOCs in general in Fig. 5*B*. A similar reaction pathway was previously established by us for halogenated fumigants (5, 6), and the abbreviated structure in Fig. 5*B* is shared by many XOCs with halide substitutions on an aliphatic carbon. Therefore, it is likely that more XOCs are potentially reactive to thiosulfate, and the rate of reaction is determined by the size and type of substitutions on the primary carbon.

Toxicological Alterations. Bioassays for acute toxicity to the luminescent bacterium *Vibrio fisheri* showed that the bacterial EC_{50} increased from 150–300 mM for the herbicides to 1,600–12,000 mM for the transformed products. Such increases in EC_{50} suggest dramatic decreases in acute toxicity. Compounds with EC_{50} of >1500 for the tester bacterium are considered nontoxic or only slightly toxic. The reaction products also were subjected to mutagenicity assay by exposure to strains of *Salmonella typhimurium*. No mutagenicity was detected in any of the reaction products, regardless of whether or not S9 activation was used before the exposure.

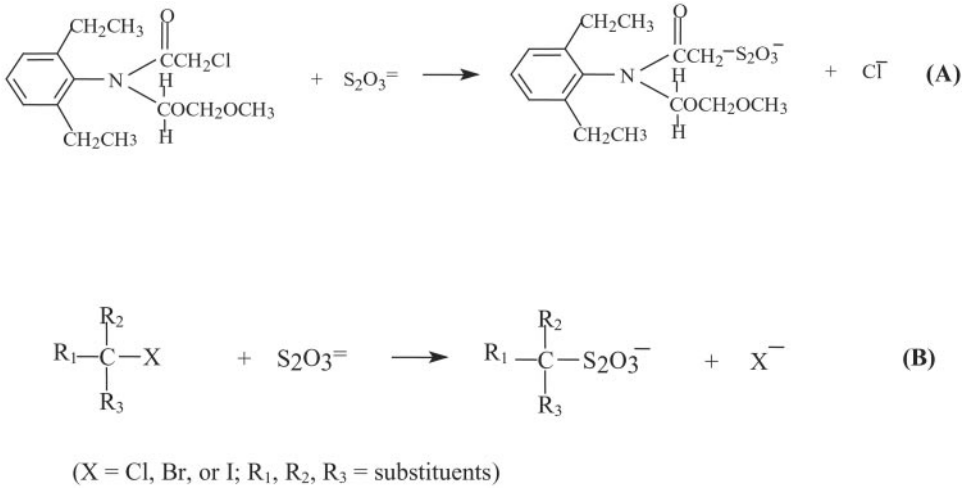

Fig. 5. The initial step of nucleophilic dehalogenation reaction between alachlor and thiosulfate salts (*A*) and halogenated compounds and thiosulfate salts (*B*).

The biological activity of XOCs often correlates with their capacity to alkylate biologically important macromolecules such as DNA and proteins (17–19). This capacity is largely attributable to the halide substitution. It seems that dehalogenation by thiosulfate stabilizes the nucleophilic center in XOCs, thus rendering these compounds biologically inactive. It is well known that enzymatically mediated *S*-glutathione conjugation is a common protective mechanism used by humans and animals against cyto- and genotoxicity of anthropogenic substances such as drugs, carcinogens, and other toxicants (20–22). Further, it is known that glutathione conjugation widely occurs in higher plants and plays a critical role in herbicide selectivity (23). A recent study showed that soil microorganisms used a similar mechanism in producing sulfide (SH^-) to degrade chloroacetanilide herbicides, and this reaction could be reproduced abiotically by sodium sulfide under reduced conditions (24). If our assays were indicative of a general reduction in human and ecological toxicity, nucleophilic substitution of XOCs by thiosulfate is a chemical reaction analogous to some biological detoxification processes and would be environmentally beneficial.

Potentially, this reaction can be incorporated in numerous scenarios, including cleanup of polluted aquifer systems, remediation of spill sites, disposal of wastes and wastewater, decontamination of containers and equipment, and control of emissions of volatile XOCs (e.g., soil fumigants and chlorinated solvents). The use of this reaction for environmental remediation has multiple advantages. The reaction seems highly specific to XOCs, in contrast to other methods that are nonselective, e.g., oxidation-based treatments and solvent flushing. Nonselective treatments tend to be destructive to the systems that are being treated. Secondly, common thiosulfate salts are commercial fertilizers and are relatively nontoxic. For instance, the LD_{50} (rat) of STS is similar to that of table salt (sodium chloride), according to the Merck Index. Previous studies have shown that in soil, thiosulfate is oxidized to sulfate with time (25). Moreover, the reaction proceeds rapidly at low thiosulfate concentrations and under ambient conditions, making its application highly suitable for environmental remediation.

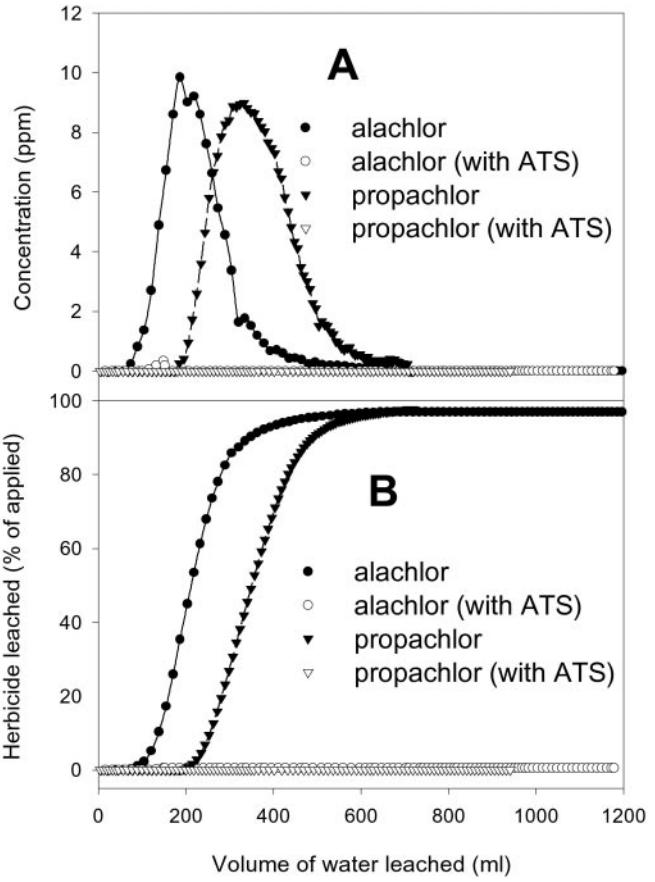

Fig. 6. Effect of ATS amendment on alachlor leaching through a sand column and propachlor leaching through a soil column. (*A*) Herbicide concentrations in leachate. (*B*) Cumulative fractions of herbicides leached through.

Herbicide Removal from Soil-Sand Systems. The rapid reaction in environmental matrices and detoxification make thiosulfate-initiated dehalogenation a versatile option for decontaminating XOCs. We tested the use of this approach for removing herbicide residues from soil and sandy aquifers—a simulation of soil/aquifer remediation. Amendment of ATS into the saturated soil/sand columns essentially prevented the herbicides from appearing in the leachate, achieving ≈100% removal of herbicides as compared with the untreated columns (Fig. 6). This simple demonstration indicates that thiosulfate salts may be introduced into polluted soil or aquifer systems to detoxify XOC contaminants that are otherwise difficult to remove. The introduction of thiosulfate salts may be easily achieved by injection through wells or by leaching in through controlled surface irrigation. Similar uses also can be devised for other susceptible halogenated contaminants.

Conclusions. We have established that thiosulfate-initiated dehalogenation can occur with a number of environmentally significant XOCs under ambient conditions and at low thiosulfate concentrations, and that the reaction causes detoxification. These findings, and the fact that common thiosulfate salts are fertilizers or otherwise readily available products, make this a reaction potentially useful for decontaminating XOCs in the environment. The specificity of this reaction clearly indicates that many more XOCs may be subject to this reaction, rendering the significance of this discovery much greater than what has already been demonstrated so far in our studies. Research should be initiated to screen contaminants that are susceptible to this reaction to obtain detailed information on human and ecological toxicities of the transformed products and to develop environment-compatible applications by using this reaction.

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